

**Patent Claims**

1. Method for finding substances which interact with the enzyme protoporphyrinogen oxidase (PPO) comprising the following steps:

a) preparing mixtures which comprise, in various concentrations, (i) PPO, (ii) a substance which is capable of interacting with PPO and which fluoresces when exposed to suitable irradiation, and (iii) a substance to be tested, or a mixture of substances to be tested,

b) irradiating the mixtures with plane-polarized light of a suitable wavelength which excites the fluorescent substance to emit light, and

c) measuring the fluorescence polarization values or the anisotropy values of the light emitted,

where a decrease in the fluorescence polarization value with an increasing concentration of the substance to be tested or of the mixture of substances to be tested indicates an interaction of one or more substances to be tested with PPO.

2. Method for assaying whether a substance interacts with the enzyme protoporphyrinogen oxidase (PPO) comprising the following steps:

a) preparing mixtures which comprise, in various concentrations, (i) PPO and (ii) a substance which fluoresces when exposed to suitable irradiation,

b) irradiating the mixtures with plane-polarized light of a suitable wavelength which excites the fluorescent substance to emit light,

- c) measuring the fluorescence polarization values or the anisotropy values of the light emitted,

where an increase in the fluorescence polarization value with a decreasing concentration of the fluorescent substance indicates an interaction with PPO.

3. Method according to Claim 1 or 2, characterized in that the PPO is a plant PPO.

4. Method according to one of Claims 1 to 3, characterized in that a PPO-containing plant cell extract is employed.

5. Method according to one of Claims 1 to 3, characterized in that biochemically purified PPO from plant cell extracts is employed.

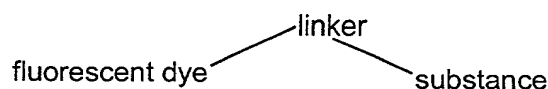
6. Method according to one of Claims 1 to 3, characterized in that recombinantly produced PPO is employed.

7. Method according to one of Claims 1 to 6, characterized in that the fluorescent substance is a substance labelled with a fluorescent dye.

8. Method according to Claim 7, characterized in that the fluorescent dye is fluorescein or a fluorescein derivative.

9. Method according to one of Claims 1 to 8, characterized in that the substance which is capable of interacting with PPO is a PPO ligand, a natural PPO substrate, a natural product of the PPO enzyme reaction or a herbicidally active PPO inhibitor.

10. Method according to one of Claims 1 to 9, characterized in that the fluorescent substance has the following structure:



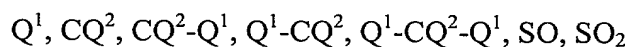
where

"linker"

5

represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:

10



15

where in each case  $Q^1$  and  $Q^2$  represent O, S or NH,

or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

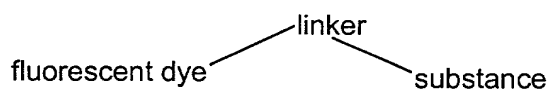
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"substance"

represents a substance which is capable of interacting with PPO.

25

11. Substance labelled with a fluorescent dye, characterized by the following structure:



where

"linker"

represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:

$Q^1$ ,  $CQ^2$ ,  $CQ^2-Q^1$ ,  $Q^1-CQ^2$ ,  $Q^1-CQ^2-Q^1$ ,  $SO$ ,  $SO_2$

where in each case  $Q^1$  and  $Q^2$  represent O, S or NH,

or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

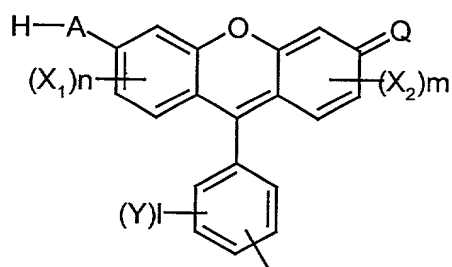
"substance"

represents a substance which is capable of interacting with PPO.

12. Substance according to Claim 11, characterized in that

"fluorescent dye"

represents a dye group which is characterized by the formula hereinbelow



where

A represents O or NH,

Q represents O, S or NH,

X<sup>1</sup> represents hydrogen, halogen, nitro, hydroxyl, carboxyl (COOH),  
sulpho (SO<sub>3</sub>H) or alkyl,

X<sup>2</sup> represents hydrogen, halogen, nitro, hydroxyl, carboxyl (COOH),  
sulpho (SO<sub>3</sub>H) or alkyl,

Y represents halogen, alkyl, carboxyl (COOH) or sulpho (SO<sub>3</sub>H),

l represents the indices 0 to 4, and

m, n represents the indices 0 to 3.

13. Substance according to Claim 12, characterized in that

A represents O.

14. Substance according to Claim 12 or 13, characterized in that

Q represents O.

15. Substance according to one of Claims 12 to 14, characterized in that

$X^1$  represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms, and

$X^2$  represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms.

16. Substance according to one of Claims 12 to 15, characterized in that

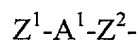
$X^1$  represents hydrogen, fluorine, chlorine, methyl or ethyl, and

$X^2$  represents hydrogen, fluorine, chlorine, methyl or ethyl.

17. Substance according to one of Claims 11 to 16, characterized in that the hydrocarbon chain of the linker has up to 10 carbon atoms.

18. Substance according to one of Claims 11 to 17, characterized in that the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms.

19. Substance according to one of Claims 11 to 18, characterized in that the substance has the following structure:



where

$A^1$  represents a single bond, O or S,

$Z^1$  represents a carbocyclic or heterocyclic group having in each case up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms, and

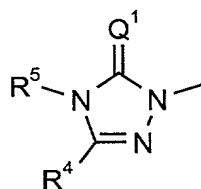
5  $Z^2$  represents a carbocyclic or heterocyclic group having in each case up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms.

20. Substance according to Claim 19, characterized in that

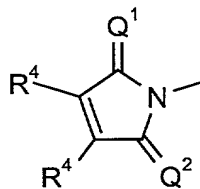
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$A^1$  represents a single bond,

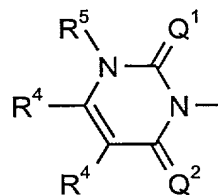
$Z^1$  represents one of the following groups



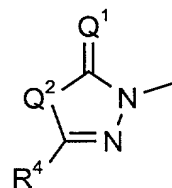
( $Z^1$ )



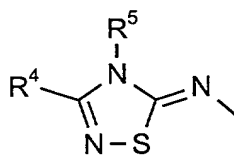
( $Z^2$ )



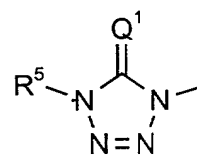
( $Z^3$ )



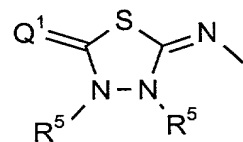
( $Z^4$ )



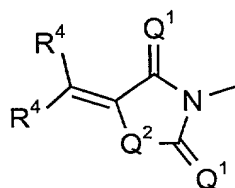
( $Z^5$ )



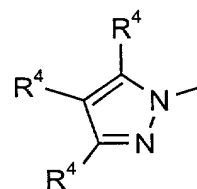
( $Z^6$ )



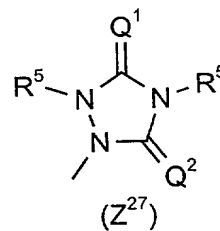
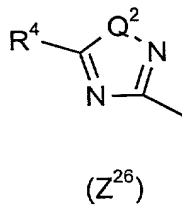
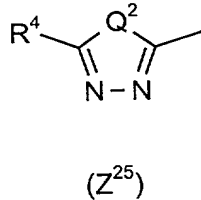
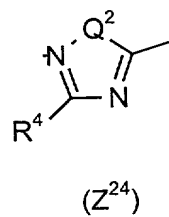
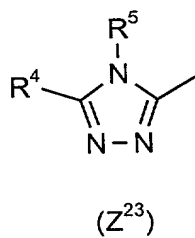
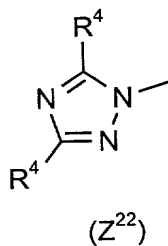
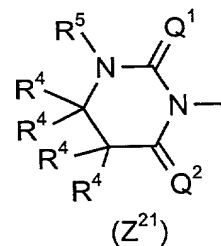
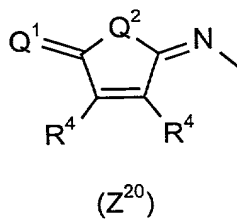
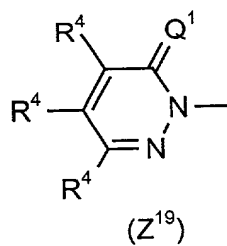
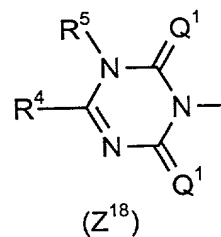
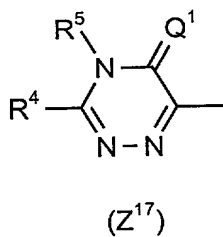
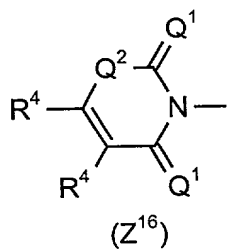
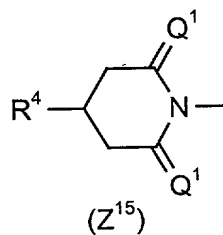
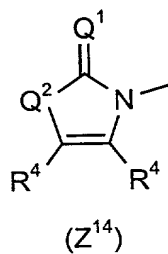
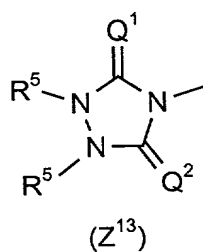
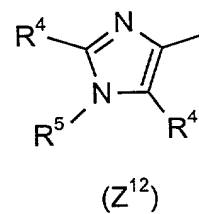
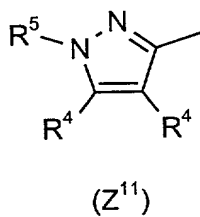
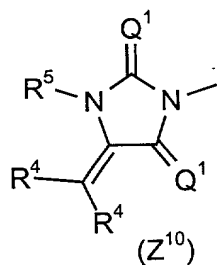
( $Z^7$ )



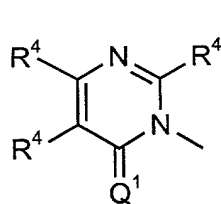
( $Z^8$ )



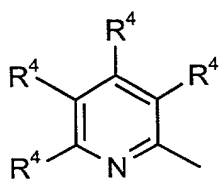
( $Z^9$ )



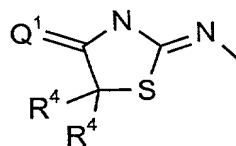




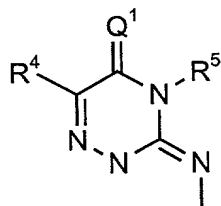
(Z<sup>28</sup>)



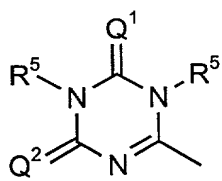
(Z<sup>29</sup>)



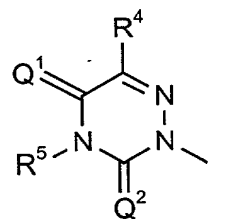
(Z<sup>30</sup>)



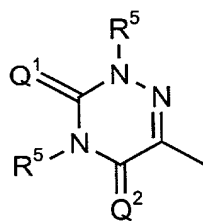
(Z<sup>31</sup>)



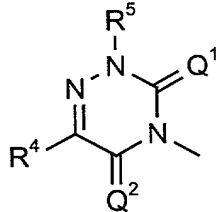
(Z<sup>32</sup>)



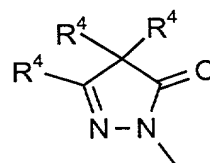
(Z<sup>33</sup>)



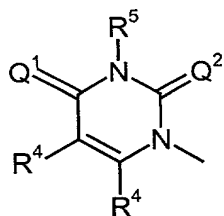
(Z<sup>34</sup>)



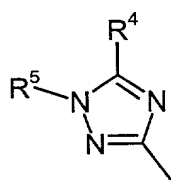
(Z<sup>35</sup>)



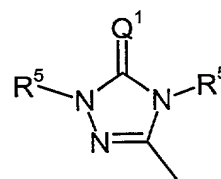
(Z<sup>36</sup>)



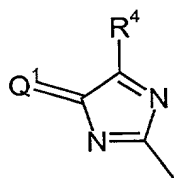
(Z<sup>37</sup>)



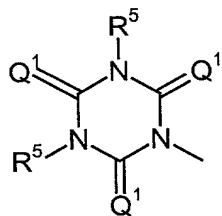
(Z<sup>38</sup>)



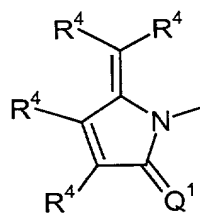
(Z<sup>39</sup>)



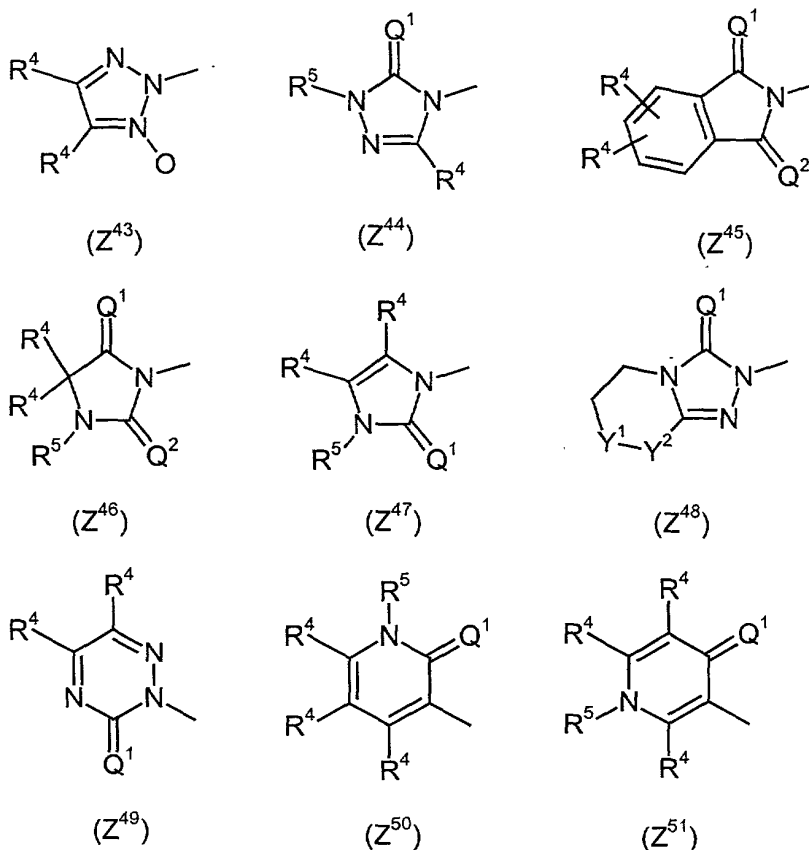
(Z<sup>40</sup>)



(Z<sup>41</sup>)



(Z<sup>42</sup>)



where

Q<sup>1</sup> represents O or S,

5      Q<sup>2</sup> represents O or S,

R<sup>4</sup> represents hydrogen, amino, nitro, cyano, carboxyl, carbamoyl, thio-  
 carbamoyl, halogen, alkyl which has 1 to 6 carbon atoms and which is  
 optionally substituted by cyano, halogen or C<sub>1</sub>-C<sub>4</sub>-alkoxy, or  
 10      represents alkenyl or alkynyl, each of which has 2 to 6 carbon atoms  
 and each of which is optionally substituted by halogen or represents  
 alkoxy or alkoxy carbonyl, each of which has 1 to 6 carbon atoms in  
 the alkyl groups and each of which is optionally substituted by cyano,  
 halogen or C<sub>1</sub>-C<sub>4</sub>-alkoxy, or represents alkenyloxy or alkynyloxy, each  
 15      of which has 3 to 6 carbon atoms and each of which is optionally

substituted by halogen, or represents alkylthio which has 1 to 6 carbon atoms which is optionally substituted by cyano, halogen or C<sub>1</sub>-C<sub>4</sub>-alkoxy, or represents alkenylthio or alkynylthio, each of which has 3 to 6 carbon atoms and each of which is optionally substituted by halogen, or represents alkylamino or dialkylamino, each of which has 1 to 6 carbon atoms in the alkyl groups, or represents cycloalkyl or cycloalkylalkyl, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by cyano, halogen or C<sub>1</sub>-C<sub>4</sub>-alkyl,

R<sup>5</sup> represents hydrogen, hydroxyl, amino, cyano, or represents alkyl, alkoxy, alkoxycarbonyl or alkylamino, each of which has up to 6 carbon atoms and each of which is optionally substituted by cyano, halogen or C<sub>1</sub>-C<sub>4</sub>-alkoxy, or represents alkenyl or alkynyl, each of which has up to 6 carbon atoms and each of which is optionally substituted by halogen, or represents cycloalkyl or cycloalkylalkyl, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by cyano, halogen or C<sub>1</sub>-C<sub>4</sub>-alkyl, or represents phenyl or phenyl-C<sub>1</sub>-C<sub>4</sub>-alkyl each of which is optionally substituted by nitro, cyano, halogen, C<sub>1</sub>-C<sub>4</sub>-alkyl, C<sub>1</sub>-C<sub>4</sub>-halogenoalkyl, C<sub>1</sub>-C<sub>4</sub>-alkoxy or C<sub>1</sub>-C<sub>4</sub>-halogenoalkoxy,

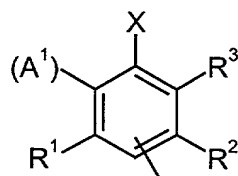
Y<sup>1</sup> represents O, S, SO, SO<sub>2</sub>, NH, N(alkyl) or methylene and

Y<sup>2</sup> represents a single bond or O, S, SO, SO<sub>2</sub>, NH or N(alkyl),

Y<sup>1</sup> and Y<sup>2</sup> being different in each individual case,

and

$Z^2$  represents the following group



5 where

$R^1$  is hydrogen, nitro, cyano or halogen,

10  $R^2$  is nitro, hydroxyl, cyano, carbamoyl, thiocarbamoyl, or represents alkyl or alkoxy, each of which has 1 to 4 carbon atoms and each of which is optionally substituted by halogen, and

15  $R^3$  represents nitro, hydroxyl, mercapto, amino, hydroxyamino, cyano, carboxyl, carbamoyl, thiocarbamoyl, halogen, or represents alkyl, alkylcarbonyl, alkoxy, alkoxycarbonyl, alkylthio, alkylsulphinyl, alkylsulphonyl or alkylamino, each of which has 1 to 6 carbon atoms in the alkyl groups and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen, C<sub>1</sub>-C<sub>4</sub>-alkoxy or C<sub>1</sub>-C<sub>4</sub>-alkoxy-carbonyl, or represents alkylsulphonylamino, N,N-bis-alkylsulphonyl-amino, N-alkylcarbonyl-N-alkylsulphonyl-amino, each of which has 1 to 4 carbon atoms in the alkyl groups and each of which is optionally substituted by halogen, or represents  
20 alkenyl, alkenyloxy, alkenylthio, alkenylamino, alkynyl, alkynyloxy, alkynylthio, alkynylamino, each of which has up to 6 carbon atoms in the alkenyl or alkynyl groups and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen or C<sub>1</sub>-C<sub>4</sub>-alkoxy-carbonyl, or represents cycloalkyl,

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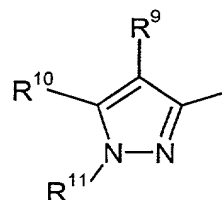
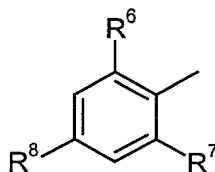
cycloalkyloxy, cycloalkylthio, cycloalkylamino, cycloalkylsulphonylamino, cycloalkylalkyl, cycloalkylalkoxy, cycloalkylalkylthio or cycloalkylalkylamino, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moieties and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen or C<sub>1</sub>-C<sub>4</sub>-alkyl, or represents aryl, aryloxy, arylthio, arylamino, arylalkyl, arylalkoxy, arylalkylthio, arylalkylamino, N-arylcarbonyl-N-alkylsulphonyl-amino, each of which has 6 or 10 carbon atoms in the aryl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by nitro, cyano, carboxyl, carbamoyl, halogen, C<sub>1</sub>-C<sub>4</sub>-alkyl, C<sub>1</sub>-C<sub>4</sub>-halogenoalkyl, C<sub>1</sub>-C<sub>4</sub>-alkoxy, C<sub>1</sub>-C<sub>4</sub>-halogenoalkoxy or C<sub>1</sub>-C<sub>4</sub>-alkoxy-carbonyl, and

X represents hydrogen or halogen.

21. Substance according to Claim 19, characterized in that

A<sup>1</sup> represents O,

Z<sup>1</sup> represents one of the following groups



where

R<sup>6</sup> represents cyano or halogen,

$R^7$  represents hydrogen or halogen,

$R^8$  represents cyano, halogen, or represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms,

5

$R^9$  represents hydrogen or halogen,

$R^{10}$  represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms, and

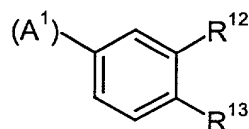
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$R^{11}$  represents alkyl having 1 to 4 carbon atoms,

and

15

$Z^2$  represents the following group



in which

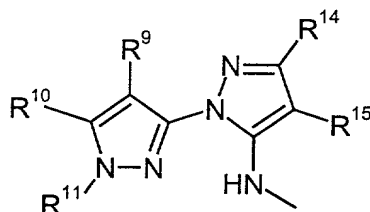
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$R^{12}$  represents carboxyl, or represents alkoxy or alkoxycarbonyl, each of which has up to 4 carbon atoms and each of which is optionally substituted by cyano, halogen or  $C_1$ - $C_4$ -alkoxy, and

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$R^{13}$  represents cyano, carbamoyl, thiocarbamoyl, halogen or represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms.

22. Substance according to Claim 19, characterized in that the substance has the following general formula:



in which

5  $R^9$ ,  $R^{10}$  and  $R^{11}$  are as defined in Claim 20,

$R^{14}$  represents hydrogen, halogen or alkyl having 1 to 4 carbon atoms, and

10  $R^{15}$  represents nitro, cyano, carbamoyl, thiocarbamoyl, or represents alkyl or alkoxy, each of which has 1 to 4 carbon atoms and each of which is optionally substituted by cyano, halogen or  $C_1$ - $C_4$ -alkoxy.

23. Assay system comprising

- 15 a) containers with mixtures as defined in one of Claims 1 to 10,
- b) a device for irradiating the mixtures of plane-polarized light of a wavelength which excites the fluorescent substance to emit light, and
- 20 c) a device for measuring the fluorescence polarization values or the anisotropy values of the light emitted.

24. Use of a substance according to one of Claims 11 to 22 for fluorescence correlation spectroscopy.

25 25. Substances which interact with the enzyme protoporphyrinogen oxidase and which have been identified by the method according to one of Claims 1 to 10.